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Benzene ADME Phenotypes and Haplotype Association Analyses

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NTP Board of Scientific Counselors
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Approach and Specific Aims

- Determine benzene ADME kinetics for a single oral exposure (Project 1: ADME and Toxicogenetics)
- Perform Haplotype Association Mapping (SNPs)*
- Identify significant QTLs and perform bioinformatic analysis using Ensembl BioMart and other databases to identify sequences associated with multigenic complex ADME phenotypes

* SNPs and CNV (insertion, deletions, indels, etc.)



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Applications of HAM

- Identify novel quantitative trait loci (QTL)
- Identify most informative strains for future QTL studies
- Narrow experimental QTL region
- Reduce the number candidate genes for further research and functional validation



HAM - Sliding Window Approach

STEP 1:

Infer local haplotypes
from 3 contiguous SNPs

CHR	POS	rs.id	129S1/SvImJ	A/J	AKR/J	BALB/cByJ	BTBR_T+/-f/J	C3H/HaJ	C57BL/6J	CAST/EJ	MOLF/EJ	NOD/LtJ	NZWLacJ	PWD/Pjh	WSBE/Eu		
1	7956353	rs31561597	A	A	A	A	A	A	T	G	T	A	A	A	G	T	
1	7956371	rs32472117	A	A	A	A	A	A	T	A	T	A	A	A	A	T	
1	7960008	rs31972217	A	A	G	A	A	G	G	G	A	A	A	G	G	G	
1	7966870	rs32048100	A	A	C	A	A	C	G	C	G	A	A	A	C	G	
1	7974686	rs31302275	T	T	T	T	T	T	G	T	G	T	T	T	T	G	
1	7998687	rs31819474	T	T	T	T	T	A	G	A	A	T	T	T	A	A	G
1	8001665	rs30642706	G	A	T	T	T	T	C	C	A	A	A	A	G	C	A
1	8002769	rs32608115	T	T	C	C	A	A	C	C	A	T	T	T	A	C	G

STEP 2:

Group strains according to
inferred haplotype groups

CHR	POS	rs.id	129S1/SvImJ	A/J	BALB/cByJ	BTBR_T+/-f/J	FVB/NJ	KK/HU	MOLF/EJ	NOD/LtJ	AKR/J	C3H/HaJ	CAST/EJ	NZWLacJ	PWD/Pjh	WSBE/Eu
1	7960008	rs31972217	A	A	A	A	A	A	A	G	G	G	G	G	G	G
1	7966870	rs32048100	A	A	A	A	A	A	A	C	C	C	C	C	G	G
1	7974686	rs31302275	T	T	T	T	T	T	T	T	T	T	T	T	G	G

STEP 3:

Compute test statistic for
genotype-phenotype association

McClurg et al. BMC Bioinformatics 7:62 (2006)



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Results - keep in perspective...

- Each kinetic parameter (AUC, Cmax, Tmax, or CL_F) is polygenic and are correlated
- The differences in rank order for each phenotype reflect the quantitative differences between each strain
- The quantitative phenotype should segregate according to their common haplotypes



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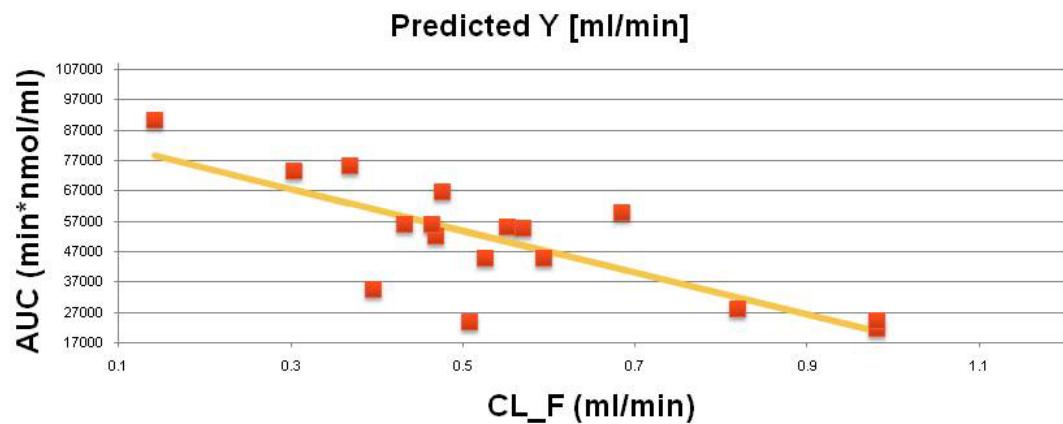
Haplotype Association Mapping – Benzene ADME

Pletcher et al. PLoS Biol 2: e393, 2004
McClurg et al..BMC Bioinformatics, 7: 63, 2006.

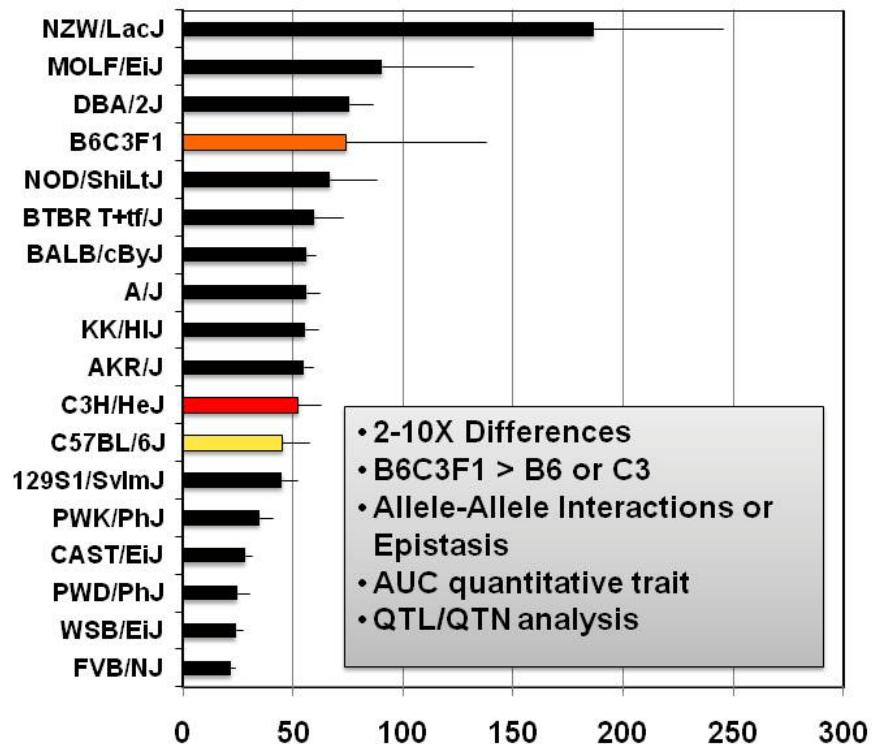


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AUC-CL_F Correlation $R^2 = 0.59$ and $p = 0.0003$

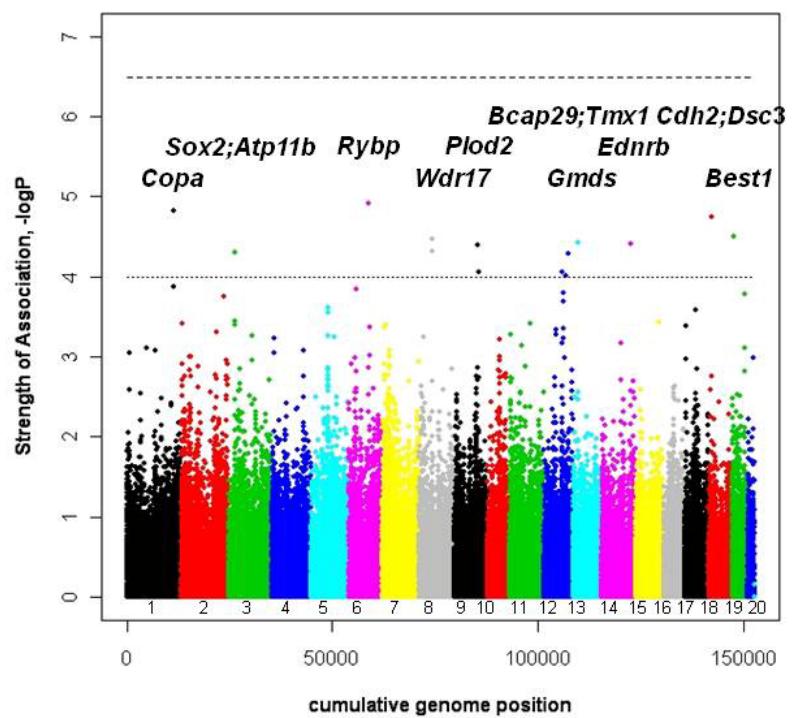


**Rank order [14C] benzene equivalents AUC (min* μ mol-Eq/ml $\times 10^3$)
following a single oral exposure (100 μ g/kg; n=5 $\mu\pm$ SD)**

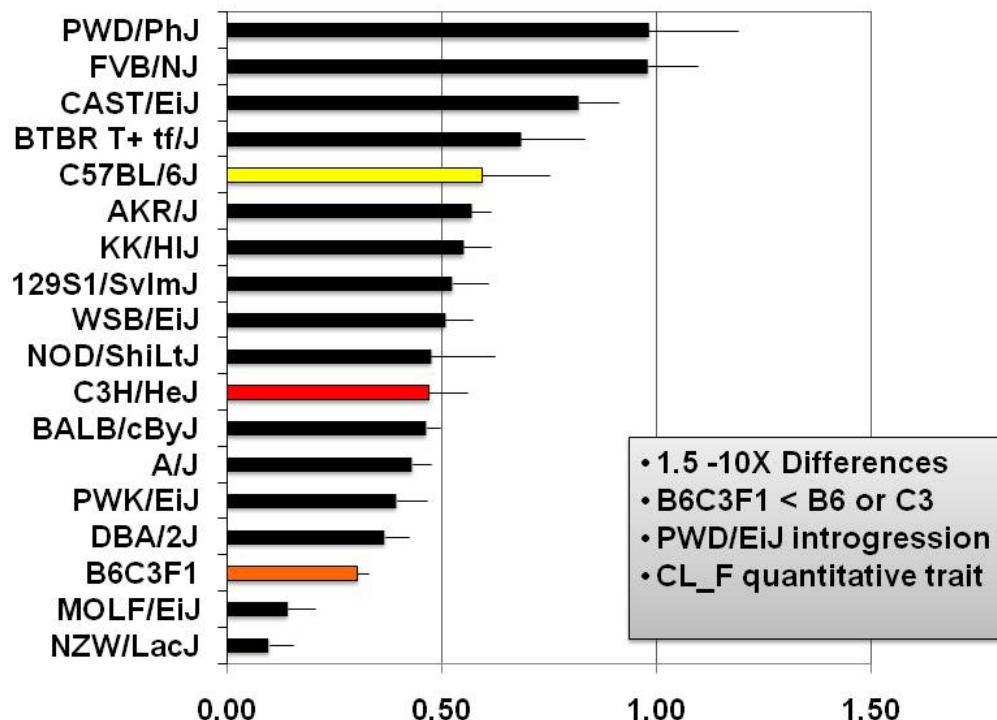




phenotype: ADME AUC

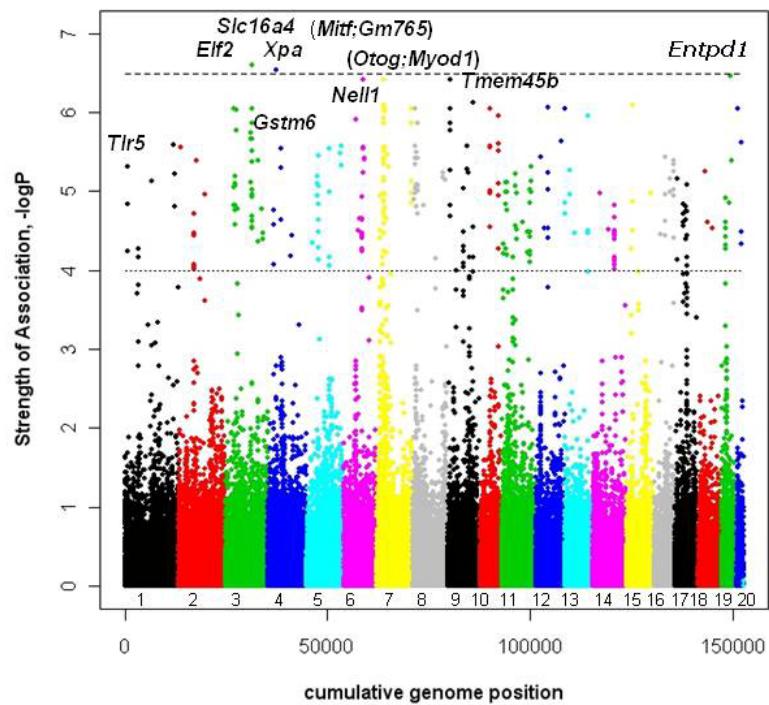


**Rank order [14C] benzene equivalents clearance rate (CL_F;
ml/min) following a single oral exposure (100 µg/kg; n=5; µ±SD)**





phenotype: ADME CL_F





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Homeostasis and Stressor Responses

ADME

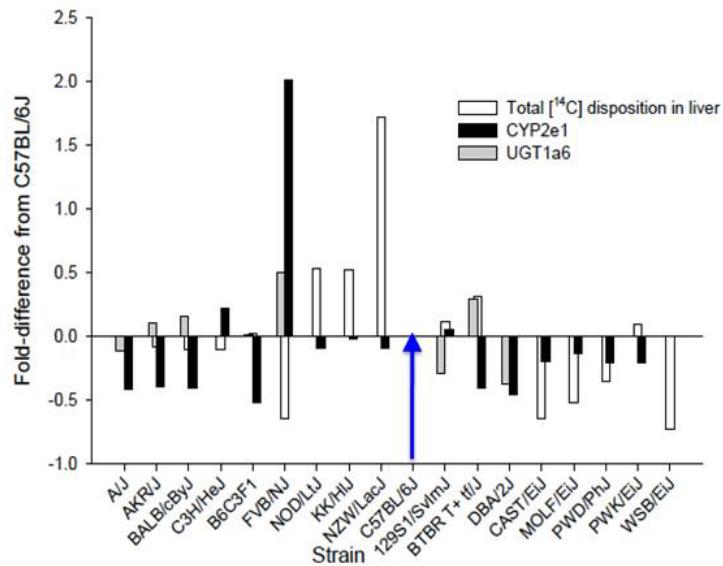
- *Ahr & Ahrr* and benzene toxicity
- 10X greater Hb-BO adducts in FVB/NJ than C57BL/6J
- Endogenous CYP substrates

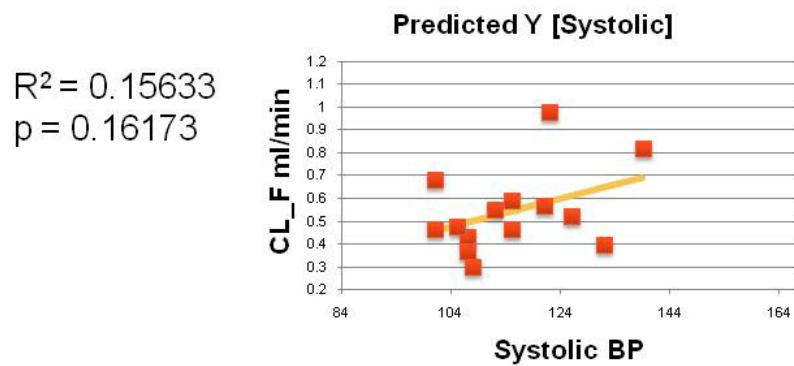
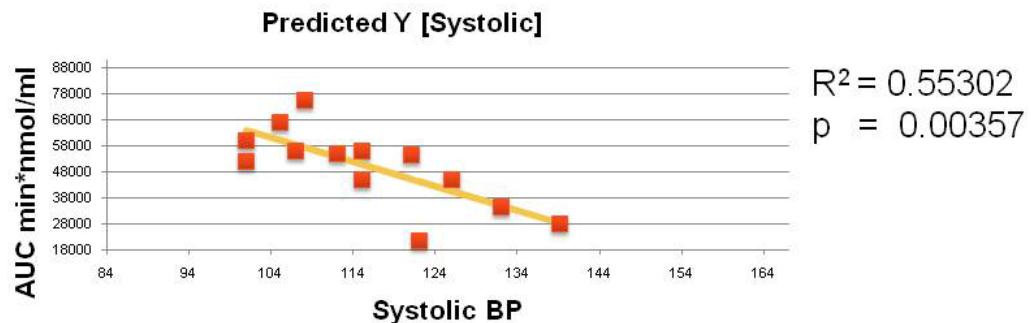
Physiological Differences

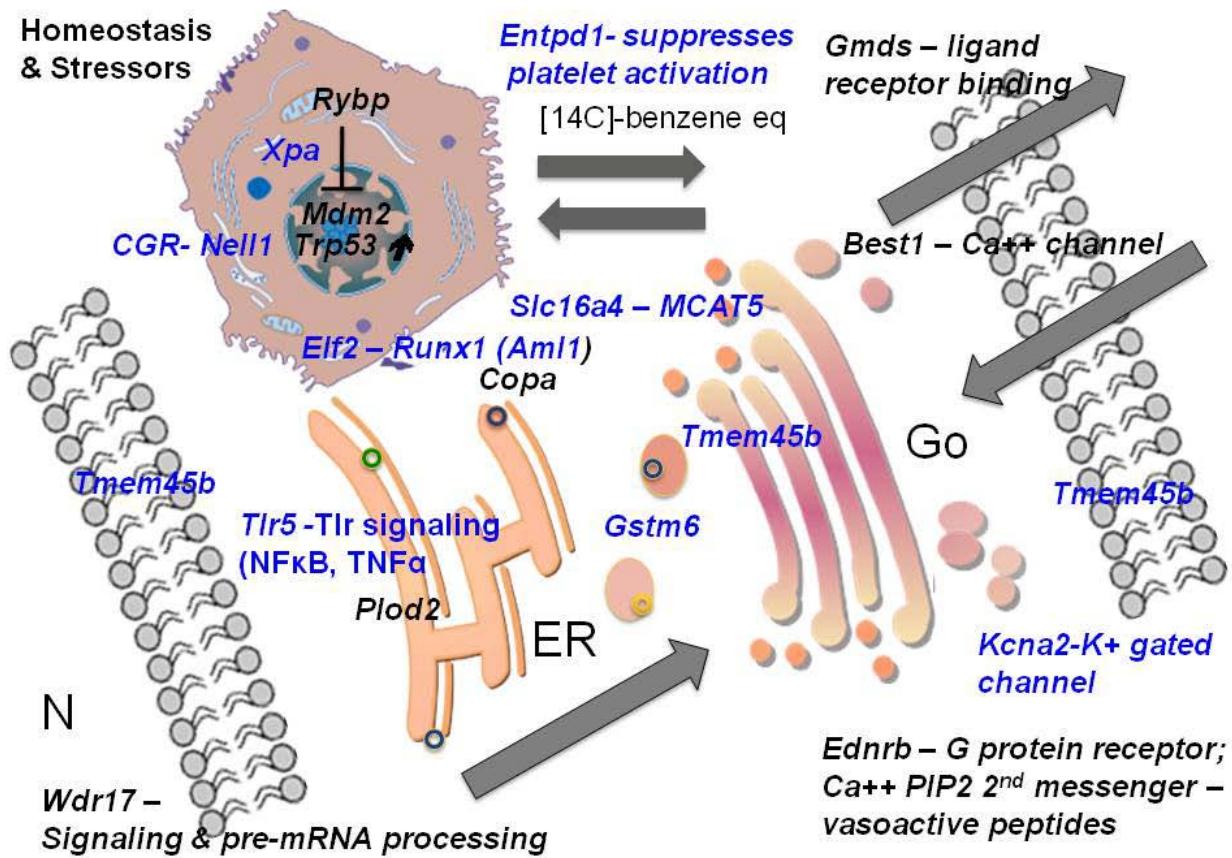
- Heart rate
- Blood pressure
- Respiration
- Kidney function

Homeostasis and Stressor Responses

Cyp2e1 and *Ugt1a6a* expression in liver 2 h post-exposure









Significance and Expected Outcomes

- ADME kinetics show quantitative or continuous trait qualities
- Epistasis observed in ADME kinetic parameters between B6, C3, and their F1 hybrid
- Both AUC and CL_F kinetic parameters are robust and a number of potential genic and intergenic sequences identified
 - ✓ AUC – ER & Golgi function and regulation, transport factors, membrane traffic, induction, signaling, DNA damage & genomic stability
 - ✓ CL-F –DNA repair, Tlr signaling (NFkB, TNF α), protein glycosylation; suppression of thrombogenesis and hematopoietic activation
- Mouse genes identified by HAM have human orthologs and highly conserved coding sequences
- Independent replication and orthologous data necessary to identify the most likely quantitative trait genes for functional validation



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Current and Future plans may include

- Complete HAM analysis of ADME kinetic parameters in 1) females & 2) male and female bone marrow ADME
- Design and perform ADME in vitro using 2D hepatocyte and/or bone marrow cultures from 34 inbred strains
- Design & perform ADME study in benzene nose only inhalation exposure study using serial sampling of 10 male mice of 34 inbred strains, if warranted
- Identify the candidate genes with largest effect and perform or facilitate functional validation (reverse phenotype studies in vitro or in vivo)



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- Glen Sipes



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Questions/Discussion



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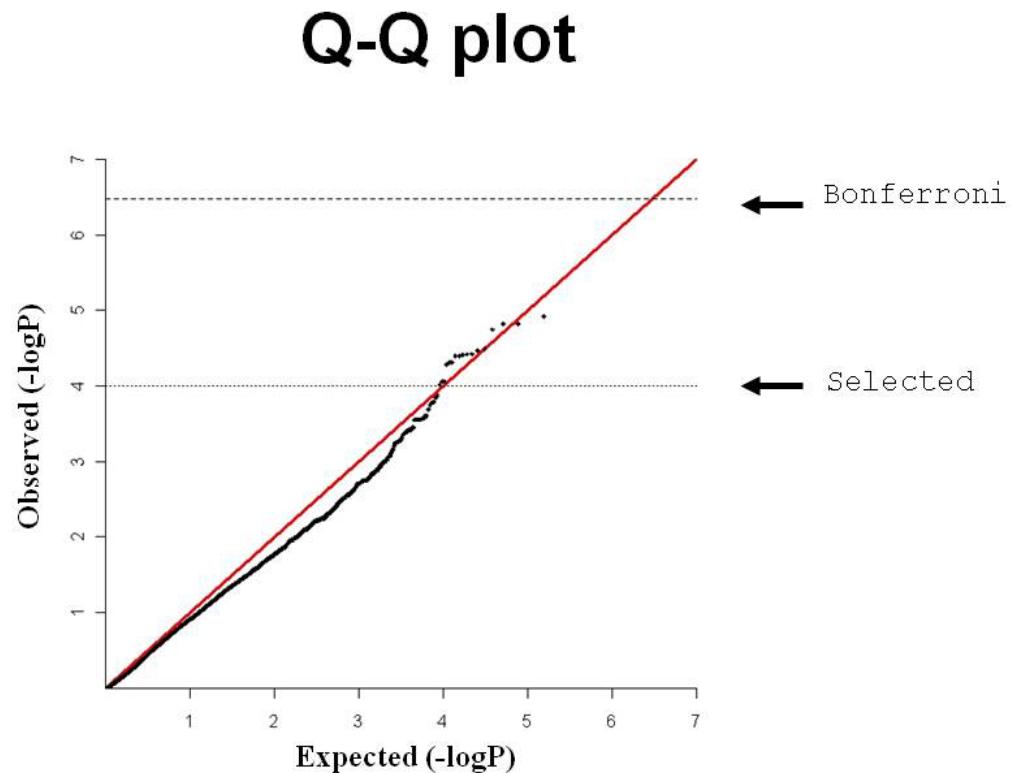
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Additional data

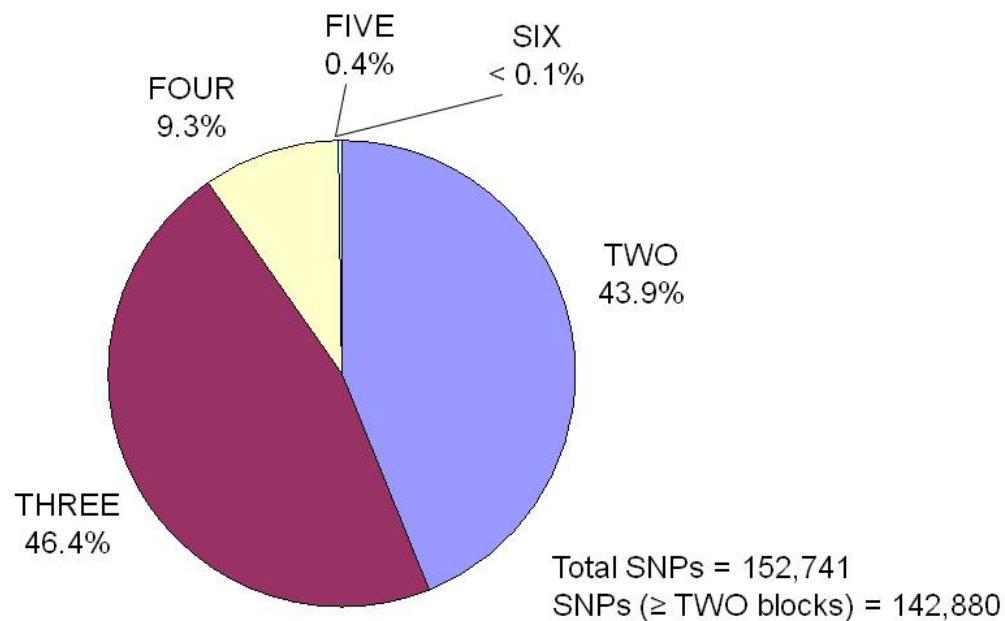


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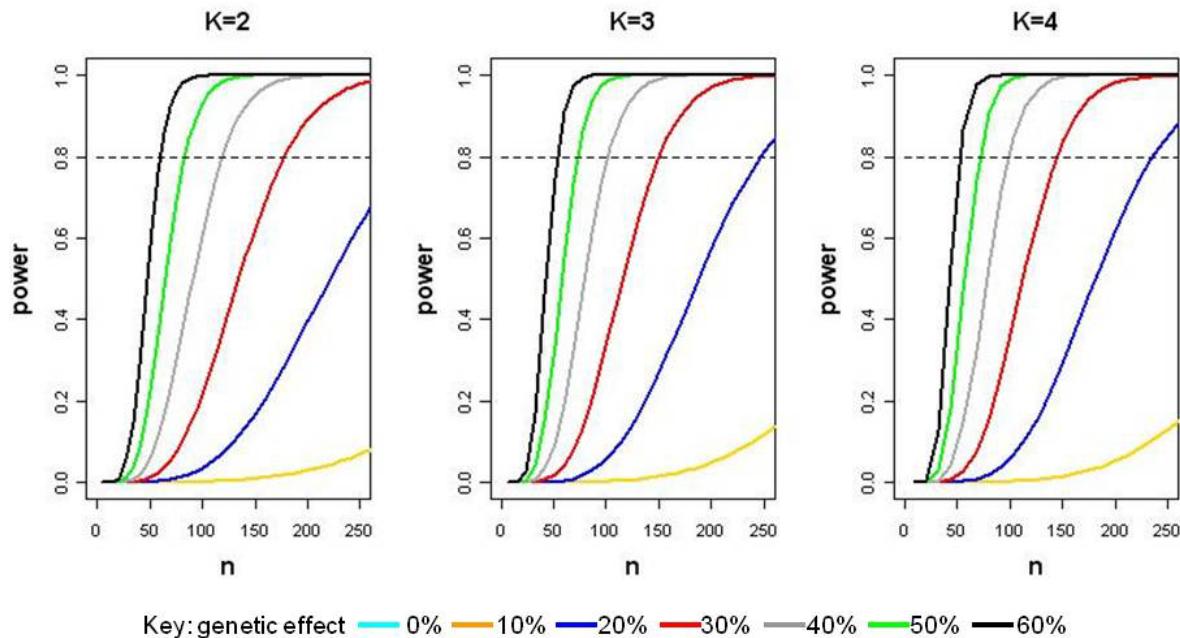




Haplotype Blocks (≥ 2 strains/block)

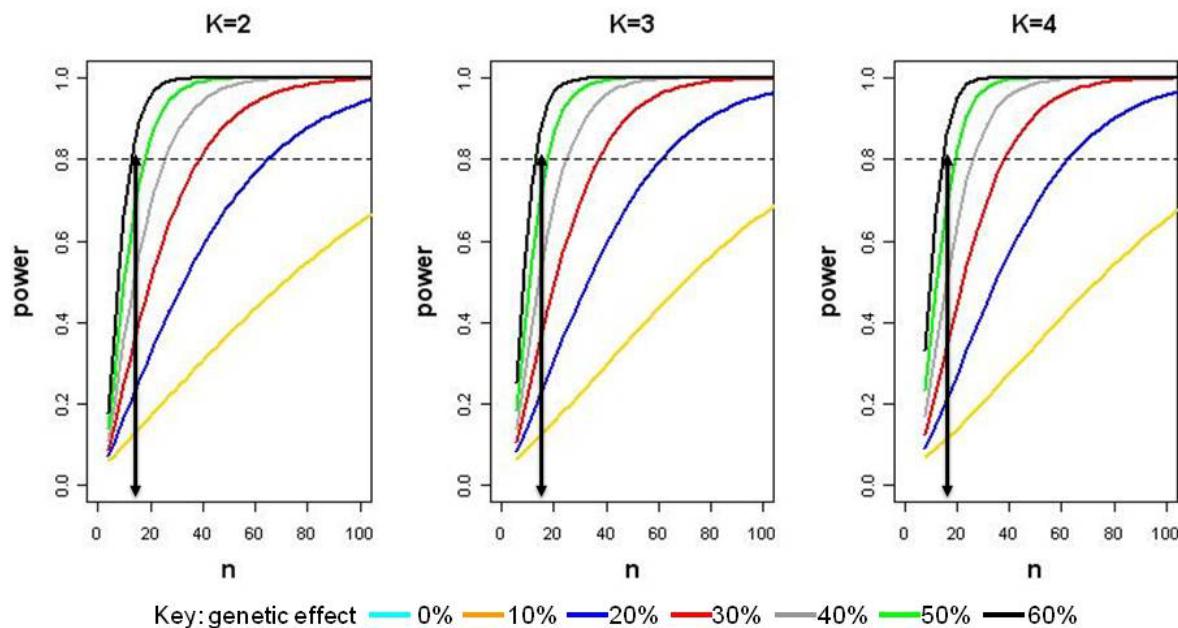


Detecting genetic effects with SNP MAF > 5%?





Detecting genetic effects with no multiple test correction?





	FVB/NJ	WSB/EiJ	PWD/PhJ	CAST/EiJ	PWK/EiJ	129S1/SvImJ	C57BL/6J	C3H/HeJ	AKR/J	KK/Hij	A/J	BALB/cByJ	BTBR T+tfJ	NOD/ShiLtJ	B6C3F1	DBA/2J	MOLF/EiJ	NZW/LacJ
FVB/NJ	0.50																	
WSB/EiJ	0.27	0.50																
PWD/PhJ	0.31	0.46	0.50															
CAST/EiJ	0.04	0.15	0.27	0.50														
PWK/EiJ	0.02	0.06	0.11	0.18	0.50													
129S1/SvImJ	0.00	0.00	0.01	0.01	0.14	0.50												
C57BL/6J	0.03	0.04	0.06	0.09	0.22	0.50	0.50											
C3H/HeJ	0.00	0.00	0.01	0.01	0.07	0.28	0.33	0.50										
AKR/J	0.00	0.00	0.00	0.00	0.00	0.12	0.22	0.41	0.50									
KK/Hij	0.00	0.00	0.00	0.00	0.01	0.14	0.23	0.40	0.47	0.50								
A/J	0.27	0.29	0.29	0.31	0.35	0.42	0.42	0.47	0.49	0.49	0.50							
BALB/cByJ	0.00	0.00	0.00	0.00	0.00	0.08	0.19	0.35	0.39	0.45	0.50	0.50						
BTBR T+tfJ	0.00	0.00	0.01	0.01	0.04	0.16	0.20	0.32	0.35	0.37	0.47	0.39	0.50					
NOD/ShiLtJ	0.02	0.02	0.02	0.03	0.07	0.16	0.18	0.26	0.28	0.30	0.43	0.31	0.39	0.50				
B6C3F1	0.21	0.22	0.22	0.24	0.27	0.33	0.33	0.37	0.38	0.39	0.42	0.39	0.42	0.46	0.50			
DBA/2J	0.00	0.00	0.00	0.00	0.00	0.01	0.03	0.06	0.04	0.06	0.37	0.05	0.18	0.36	0.49	0.50		
MOLF/EiJ																		
NZW/LacJ	0.00	0.00	0.00	0.00	0.01	0.01	0.01	0.01	0.01	0.01	0.06	0.01	0.02	0.03	0.10	0.03	0.50	



	NZW/LacJ	MOLF/Eij	B6C3F1	DBA/2J	PWK/Eij	A/J	BALB/cByJ	C3H/HeJ	NOD/ShiLtJ	WWB/Eij	129S1/SvImJ	KK/Hij	AKR/J	C57BL/6J	BTBR T+ tfj	CAST/Eij	FVB/NJ	PWD/Phj
NZW/LacJ	0.5																	
MOLF/Eij	0.47	0.5																
B6C3F1	0.22	0.41	0.5															
DBA/2J	0.00	0.36	0.4	0.5														
PWK/Eij	0.00		0.35	0.37	0.38	0.5												
A/J	0.22	0.35	0.4	0.44	0.47	0.5												
BALB/cByJ	0.00	0.31	0.27	0.06	0.19	0.47	0.5											
C3H/HeJ	0.00	0.31	0.28	0.17	0.27	0.47	0.49	0.5										
NOD/ShiLtJ	0.01	0.31	0.28	0.25	0.31	0.46	0.47	0.48	0.5									
WSB/Eij	0.00	0.29	0.22	0.05	0.12	0.43	0.27	0.36	0.42	0.5								
129S1/SvImJ	0.00	0.28	0.21	0.05	0.12	0.42	0.24	0.32	0.39	0.43	0.5							
KK/Hij	0.00	0.26	0.18	0.01	0.05	0.39	0.11	0.23	0.32	0.32	0.4	0.5						
AKR/J	0.00	0.25	0.16	0.00	0.02	0.38	0.03	0.16	0.27	0.21	0.32	0.41	0.5					
C57BL/6J	0.00	0.25	0.17	0.09	0.12	0.36	0.21	0.24	0.29	0.31	0.35	0.4	0.44	0.5				
BTBR T+ tfj	0.00	0.21	0.1	0.02	0.04	0.29	0.07	0.11	0.16	0.14	0.17	0.2	0.23	0.34	0.5			
CAST/Eij	0.00	0.15	0.03	0.00	0.00	0.19	0.00	0.00	0.02	0.00	0.01	0.01	0.01	0.11	0.22	0.5		
FVB/NJ	0.00	0.1	0.01	0.00	0.00	0.11	0.00	0.02	0.06	0.14	0.5							
PWD/Phj	0.00	0.11	0.02	0.00	0.00	0.13	0.01	0.01	0.02	0.02	0.02	0.02	0.03	0.07	0.12	0.24	0.5	0.5